18

## CLAIMS

1. A process for the synthesis of lipid cations having general formula (6):

$$\begin{array}{c|c}
O & R_1 \\
O & R_1
\end{array}$$

$$\begin{array}{c|c}
O & R_1
\end{array}$$

$$\begin{array}{c|c}
P & R_2
\end{array}$$

$$\begin{array}{c|c}
R_4 & R_3
\end{array}$$

in which:  $R_1$  represents a lipophilic chain, preferably selected from  $C_1$ - $C_{24}$  alkyl,  $C_1$ - $C_{24}$  alkenyl,  $C_1$ - $C_{24}$  alkynyl,  $C_1$ - $C_{24}$  alkanoyl, and  $C_1$ - $C_{24}$  alkenoyl or alkynoyl radicals,  $R_2$ ,  $R_3$ ,  $R_4$ , which are identical or different from one another, represent  $C_1$ - $C_{10}$  alkyl,  $C_1$ - $C_{10}$  alkenyl, or  $C_1$ - $C_{10}$  alkynyl radicals, optionally containing hydroxyl, ether, halogen and acyloxy functions, and  $X^-$  is an oxy-anion or a halide, characterized in that a compound of formula (2),

$$OR_5$$
 $OR_6$ 
 $OTs$ 
 $(2)$ 

in which  $R_5$  and  $R_6$ , which are identical or different from one another, represent a  $C_1$ - $C_5$  acyl, a benzyl group or a diolprotective group, is reacted in an alcoholic solvent with from 1 to 6 equivalents of  $NR_2R_3R_4$ , in which  $R_2$ ,  $R_3$  and  $R_4$  have the meanings given above, to give the compound of formula (3)

PCT/EP2004/052788

OR5
$$OR_{6}$$

$$R_{2}^{\oplus}$$

$$R_{3}^{\oplus}$$
OTs

WO 2005/049549

(3)

in which  $R_2$ ,  $R_3$ ,  $R_4$ ,  $R_5$  and  $R_6$  have the meanings given above.

- 2. A process according to Claim 1, characterized in that the alcoholic solvent is selected from ethanol, isopropanol and/or methanol.
- 3. A process according to Claim 1, characterized in that the alcoholic solvent is used in a quantity of from 0.5 1.5 litres per equivalent of  $NR_2R_3R_4$ .
- 4. A process according to Claim 1, characterized in that all three of  $R_2$ ,  $R_3$  and  $R_4$  are methyl radicals.
- 5. A process according to Claim 1, characterized in that it is carried out at a temperature of from 50 100°C.
- 6. A process according to Claim 1, characterized in that the diol-protective group is a ketal, preferably a cyclic ketal, even more preferably a solketal.
- 7. A process according to any one of the preceding claims, characterized in that the compound of formula (2) is obtained by reacting from 0.9 to 1.2 equivalents of compound of formula (1)

20

$$OR_5$$
 $OR_6$ 
 $OH$ 
 $(1)$ 

with 1 equivalent of tosyl halide, preferably chloride, in an apolar, organic solvent.

- 8. A process according to Claim 7, characterized in that the apolar, organic solvent is a hydrocarbon, preferably toluene.
- 9. A process according to Claim 7, characterized in that the reaction is carried out at a temperature of 15-35°C, preferably 20-25°C.
- 10. A process according to Claim 7, characterized in that the reaction is carried out with the use of from 0.8 to 1.2 litres of solvent per equivalent of compound (1).
- 11. A process according to any one of the preceding claims comprising the removal of the  $R_{\mbox{\scriptsize 5}}$  and  $R_{\mbox{\scriptsize 6}}$  groups to give compound (4)

OH
OH
$$R_2 \stackrel{\Theta}{R_4} \stackrel{\Theta}{OTs}$$

$$R_2 \stackrel{R_3}{R_3} \qquad (4)$$

in which  $R_2$ ,  $R_3$  and  $R_4$  have the meanings given above, the subsequent reaction of compound (4) with 2-4 equivalents of  $R_1$ COCl in an aprotic, apolar, organic solvent, preferably a

PCT/EP2004/052788

chlorinated solvent, in which  $R_1$  has the meaning given above, to give compound (5)

OCOR<sub>1</sub>

$$-OCOR_1$$

$$R_2 R_3 \Theta OTs$$

$$R_2 R_3 \qquad (5)$$

WO 2005/049549

in which  $R_1$ ,  $R_2$ ,  $R_3$  and  $R_4$  have the meanings given above, and the subsequent ion exchange of the tosylate anion of compound (5) with a halide anion to give the lipid cation of formula (6).

- 12. A process according to Claim 11, characterized in that groups  $R_5$  and  $R_6$  are removed by acid hydrolysis.
- 13. A process according to Claim 11, characterized in that the aprotic, apolar, organic solvent is used in a quantity of 3.5-5.5 litres per equivalent of compound (4).
- 14. A process according to Claim 11, characterized in that the aprotic, apolar, organic solvent is selected from methylene chloride, chloroform, and tetrachloroethylene.
- 15. A process according to claim 11, characterized in that the ion exchange is performed by chromatography on ion-exchange resin.
- 16. A process according to Claim 15, characterized in that the ion-exchange resin is a strong basic resin.

22

17. A process according to any one of the preceding claims, characterized in that the lipid cation of formula (6) is purified by crystallization, preferably from acetonitrile.

18. A process according to any one of the preceding claims, characterized in that the lipid cation of formula (6) is N-[1-(2,3-dioleyloxy-propyl]-N,N,N-trimethylammonium chloride (DOTAP-Cl).

## 19. A compound of formula (4)

OH
OH
$$R_2 \stackrel{\mathfrak{S}}{R_3} = OTs$$

$$R_2 \stackrel{\mathfrak{S}}{R_3} \qquad (4)$$

in which  $R_2$ ,  $R_3$  and  $R_4$ , which are identical or different from one another, represent  $C_1-C_{10}$  alkyl radicals,  $C_1-C_{10}$  alkenyl radicals, or  $C_1-C_{10}$  alkynyl radicals, optionally containing hydroxyl, ether, halogen, and acyloxy functions.

## 20. A compound of formula (5)

OCOR<sub>1</sub>

$$-OCOR_1$$

$$R_2 R_3 OTs$$

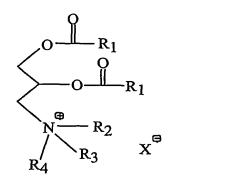
$$R_2 R_3 (5)$$

in which:  $R_1$  represents a lipophilic chain, preferably selected from  $C_1$ - $C_{24}$  alkyl,  $C_1$ - $C_{24}$  alkenyl,  $C_1$ - $C_{24}$  alkynyl,  $C_1$ - $C_{24}$  alkanoyl, and  $C_1$ - $C_{24}$  alkenoyl or alkynoyl radicals, and

23

 $R_2$ ,  $R_3$ ,  $R_4$ , which are identical or different from one another, represent  $C_1$ - $C_{10}$  alkyl,  $C_1$ - $C_{10}$  alkenyl, or  $C_1$ - $C_{10}$  alkynyl radicals, optionally containing hydroxy, ether, halogen and acyloxy functions.

21. Use of compounds of formula (4) and/or (5) as intermediates in the synthesis of cationic lipids having general formula (6):



(6)

in which  $R_1$  represents a lipophilic chain, preferably selected from  $C_1$ - $C_{24}$  alkyl,  $C_1$ - $C_{24}$  alkenyl,  $C_1$ - $C_{24}$  alkynyl,  $C_1$ - $C_{24}$  alkanoyl, and  $C_1$ - $C_{24}$  alkenoyl or alkynoyl radicals,  $R_2$ ,  $R_3$ ,  $R_4$ , which are identical or different from one another, represent  $C_1$ - $C_{10}$  alkyl,  $C_1$ - $C_{10}$  alkenyl, or  $C_1$ - $C_{10}$  alkynyl radicals, optionally containing hydroxyl, ether, halogen and acyloxy functions, and  $X^-$  is an oxy-anion or a halide.